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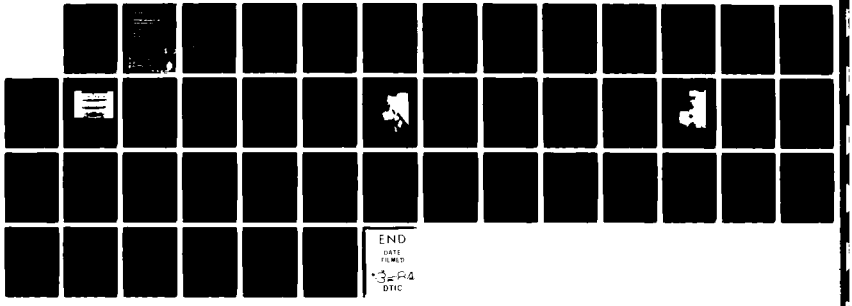

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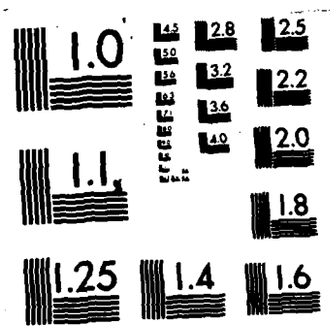

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NOTICES

This final report was submitted by Georgia Institute of Technology, Atlanta Georgia, under contract F33615-81-K-0620, job order 7757-03-08, with the USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks Air Force Base, Texas. James H. Merritt (USAFSAM/VEP) was the Laboratory Project Scientist-in-Charge.

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

James H. Merritt
JAMES H. MERRITT, M.S.
Project Scientist

John C. Mitchell
JOHN C. MITCHELL, M.S.
Supervisor

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A unique 435-MHz radiofrequency radiation exposure facility--constructed at the Georgia Institute of Technology under U.S. Air Force sponsorship--has been operationally evaluated for conducting long-term animal studies. The exposure device is a 4-tier, 12-ft-diam., circular, parallel-plate waveguide arrangement. Slotted-cylinder antennas feed the waveguide and generate a horizontally polarized field. An identical unpowered system is used for sham-exposure control. The facility consists of: the waveguides, located in separate rooms; and a suite of support rooms, for animal preparation, data collection, and cage washing. The facility can house 100 exposed rats and 100 sham-exposed rats. The triple purpose of this study has been to evaluate the system, integrate several subsystems, and train technologists in operating the system. All exposed and sham-exposed animals were anesthetized (Nembutal) and implanted with plastic cannulas in the aortic arch and exteriorized at the back of the neck. The animals were maintained in the exposure system for 8 weeks at 1 mW/cm^2 (pulse repetition rate = 100 Hz; pulse width = 1 μs). Blood was drawn, via the cannula, at weekly intervals; and ACTH, corticosterone, and

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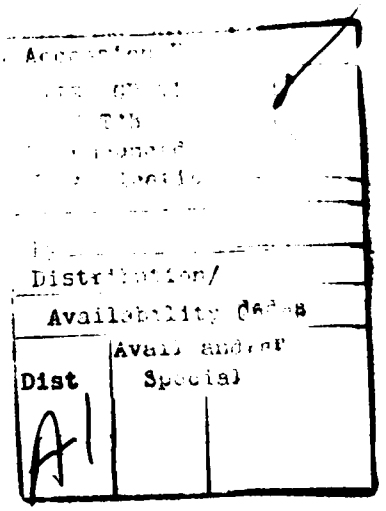
prolactin were assayed. In addition, animal weights were determined. Implantations and assays were conducted at Emory University School of Medicine.



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OPERATIONAL EVALUATION OF A NEW 435-MHz RADIOFREQUENCY RADIATION FACILITY

I. INTRODUCTION

During the last ten years, a significant amount of research has been concerned with whether biological effects, hazardous or otherwise, are induced in living systems by exposure to radiofrequency radiation (RFR). In much of this research, the engineers or biologists have necessarily exposed small animal populations to specific pulsed- or continuous-wave radiation environments for relatively short periods, during which biological endpoints (presumed to be sensitive to the radiation insult) were monitored . Although these research efforts have been helpful, in a real sense they have raised more questions than they have answered. Among the numerous reasons for this situation are the following:

- In many instances, the persons conducting the research have been qualified in engineering or biology, but not in both; consequently, key aspects of one discipline or the other were not adequately considered.
- The animal populations have often been too small to yield statistically significant data.
- Exposure facilities have been so limited that difficulty has been encountered in studying the same biological endpoints during exposure to radiation with the same propagation mode but different electromagnetic parameters.
- No rationale explains why the selected endpoints should be responsive to RFR. Also, the procedures used in accessing these endpoints have been capable of masking any radiation-induced bioeffects.
- Dosimetry determinations have not been adequate to characterize accurately the absorbed dose of radiation.
- The exposure frequency and animal species were such that a meaningful extrapolation of the data to the man-model was not possible.

To overcome such difficulties, the USAF School of Aerospace Medicine (USAFSAM), Radiation Sciences Division, is sponsoring bioeffects research programs. These involve interdisciplinary teams which expose large animal populations to real-world radiation environments, in facilities versatile enough to generate both pulsed- and continuous-wave fields. The exposure frequency and the animal species are selected to assure extrapolation of the resulting data to the man-model. The endpoints monitored are stress-sensitive, and are therefore expected to be responsive to effects induced by such environmental insults as RF radiation.

--I--

The USAFSAM-sponsored program in the Biomedical Research Division at the Georgia Institute of Technology (Georgia Tech), Engineering Experiment Station, involved four (a - d) studies.

a. Several potentially satisfactory facility concepts were analyzed theoretically, and the circular parallel-plate concept in a multi-tier configuration was identified as being the most technically and economically feasible.

b. The second program involved construction of a prototype, single-tier set of circular, parallel plates, and development of a slotted cylinder antenna to feed the plates. The plates and antenna were then evaluated by having the radiation patterns, in an anechoic chamber, measured as a function of: plate separation, frequency, radial position from the plate center, vertical position between the plates, slot parameters, etc.

c. The third program provided a full-scale 435-MHz circular parallel-plate RFR facility. The facility consisted of two 4-tier stacks of circular, parallel plates located in adjoining absorber-lined rooms. Also provided were an assay room, transmitter room, utility room, storage room, computer room, and office area--all colocated with the rooms housing the circular, parallel plates. Each tier of circular, parallel plates accommodated 25 rodents; therefore, one 4-tier configuration could be used to expose 100 rodents to a 435-MHz environment, while the other configuration could house 100 control rodents. This program provided both the Plexiglas cages for the rodents, and a 435-MHz transmitter with pulse- and continuous-wave outputs of 5 kW and 200 W, respectively.

d. The fourth program (described in this report) had, as its primary objective, the performance of a "shakedown" evaluation to familiarize project personnel with the operation of the exposure facility and the analysis of the blood samples. This program was undertaken by an interdisciplinary team of engineers from Georgia Tech and of medical professionals from Emory University. In addition to performing the shakedown evaluation, this team also integrated several remaining subsystems into the facility, and trained biomedical technologists in both cannulation techniques and microassay procedures. Efforts undertaken during this program are described in detail in this report.

II. DESCRIPTION OF THE RFR FACILITY

A description of the Radiofrequency Radiation (RFR) Facility will be particularly helpful, both here and in that section of the report in which procedures for handling the experimental animals are presented. As a key part of the Facility, characteristics of the exposure field in the Radiation Room are also given.

A. Information on the RFR Facility

The RFR Facility consists of eight rooms in the basement of the Baker Building, on the Georgia Tech main campus. These rooms were specifically designed as an essentially self-contained facility, for bioeffects studies involving large rodent populations and long-term RFR exposures. The Baker Building is a three-story brick building in which various electronic research programs are conducted; however, this is the only program involving experimental animals. Heating and airconditioning systems for the RFR Facility are isolated from others in the Baker Building, and exhaust air is routed outside through a large plenum. Power for lights, in the rooms for the exposed and sham-exposed animals, is routed through a timer that can be programmed to provide any desired lighting cycle. Hot- and cold-water supplies are taken from primary sources, and are therefore unaffected by service outages which might cause loss of water in other Baker Building locations. The construction of rooms housing the exposed and sham-exposed animals includes electromagnetic shielding to assure that stray radiation does not reach other research areas. For this purpose, aluminum-backed Sheetrock has been utilized in the walls in the Control and Radiation Rooms. This aluminum backing is joined, with conductive tape, along all seams between adjacent panels of Sheetrock; thus, an electrically conductive aluminum shield is provided for the walls. For the ceilings, individual 2-ft by 2-ft acoustical tiles have been removed from their metal support frames, covered with aluminum shielding, and remounted. The facility floor is on ground level, and therefore requires no shielding.

The RFR Facility floor plan (Rooms A - H) is shown in Figure 1. During the shakedown evaluation, Rooms A and B (Fig. 1) housed the exposed and sham-exposed rats, respectively. Identical 12-ft-diam. circular parallel-plate structures were in both rooms. These structures consisted of 4-tier stacks of circular aluminum plates, between each of which an 18-in. distance was maintained by 1.5-in.-diam. plastic rods [2]. These plates functioned as open-ended waveguides and were fed with slotted cylinder antennas at the plate centers [3]. The walls of both rooms were also lined identically with pyramid-shaped microwave-absorbing material that provided a -30 dB reflectivity at 500 MHz. The timers were used to cycle the lighting on a 6:00 a.m. - 6:00 p.m. schedule. Temperature in these rooms was maintained at 70± 2°C during the first four weeks of the evaluation, and was then increased to 74±2°C during the remaining weeks. Humidity was maintained at 48±2% throughout the evaluation. Room C (Fig. 1) was used as a storage and maintenance area. Stored items included rat chow, Styrofoam soil trays, custodial supplies, extra cages, etc. The work area in this room was used

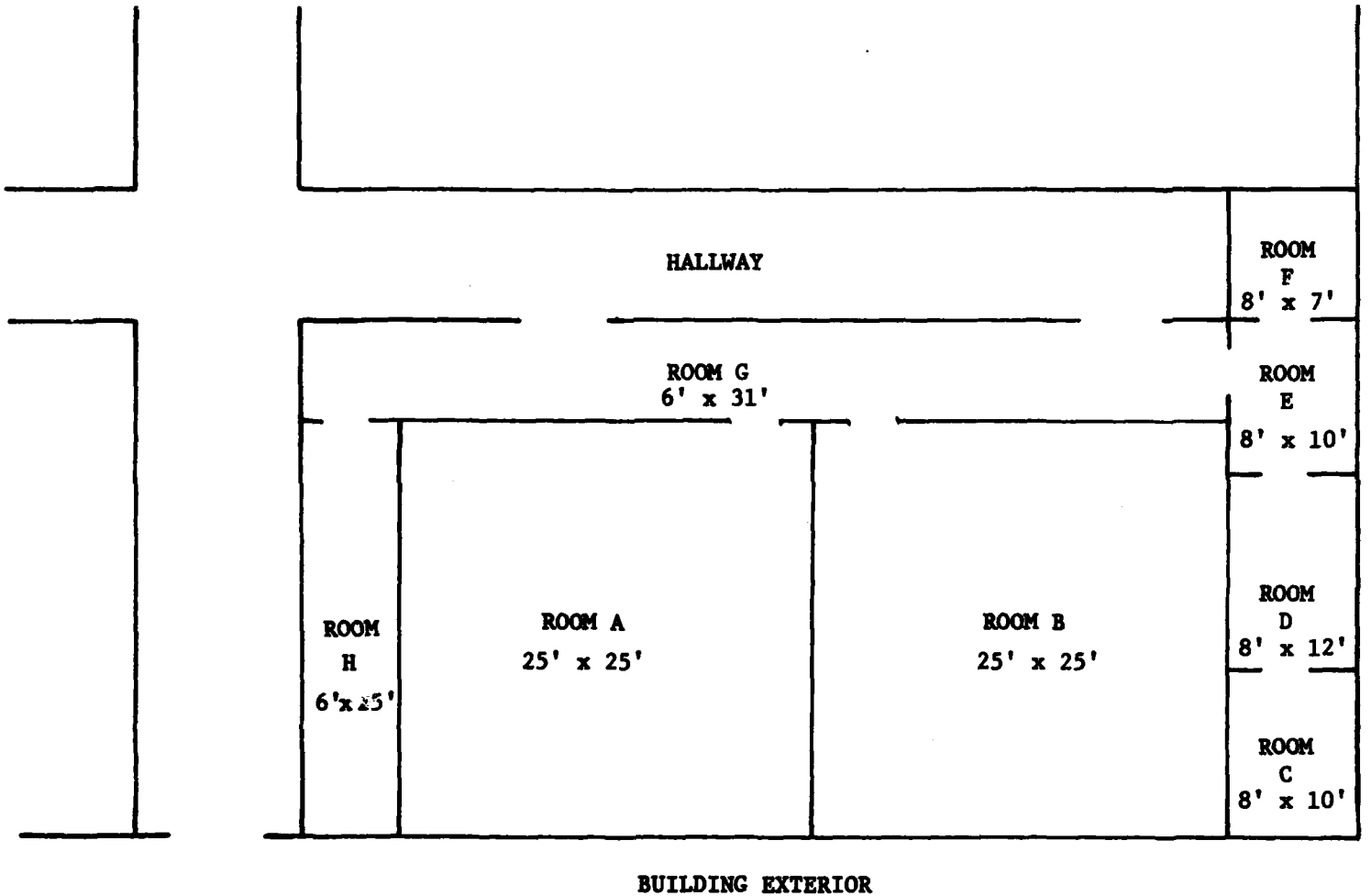

Figure 1. Floor plan for the Radiofrequency Radiation (RFR) Facility.

primarily for repairing damaged cages. Housed in Room D was the cage washer, with its entry and exit tables. Room E was the area to which the rats were transferred, from their large Plexiglas exposure cages into small Plexiglas holding cages, in preparation for the drawing of microsamples of their blood. This room also housed the electronic balance, and was therefore used when the rats were weighed. After transfer into the small Plexiglas cages, the rats were moved into Room F where an acclimation period, of 15-min or more, was provided before microsamples of blood were drawn. During this acclimation period, the door to Room F was closed and only the biological technician remained in the room. A buffer area, between the Control and Radiation Rooms and the routine activity in the hallway outside the Facility, was provided by Room G. It also contained: the Data Acquisition System into which the weight data for each animal was entered; and the desk space for the engineering technician. The 435-MHz transmitter, plus spare parts and a work bench, were in Room H. Coaxial connectors on the transmitter top provided power output ports for the four antennas located in the Radiation Room (A). Cables joined to these four connectors were routed up through the ceiling of Room H and across to the slotted cylinder antennas in Room A.

The foregoing description shows the RFR Facility to be a complex of eight contiguous rooms which provide space for:

- o housing large populations of exposed and sham-exposed rats in two identical and adjoining rooms;
- o storage of supplies and spare parts, and for maintenance of equipment and cages;
- o a commercial cage washer;
- o transfer of rats from exposure cages into holding cages;
- o weighing the rats;
- o drawing microsamples of blood from the rats;
- o a buffer zone between the Control and Radiation Rooms and routine activity in the Baker Building; and
- o housing and operating the 435-MHz transmitter.

B. Exposure Field Description

Slotted-cylinder antennas were selected to feed the circular, parallel-plate waveguides because they generate a horizontally polarized field with an essentially constant amplitude pattern. Performance of these antennas can be intuitively understood by referring to Figure 2, where the antenna is first

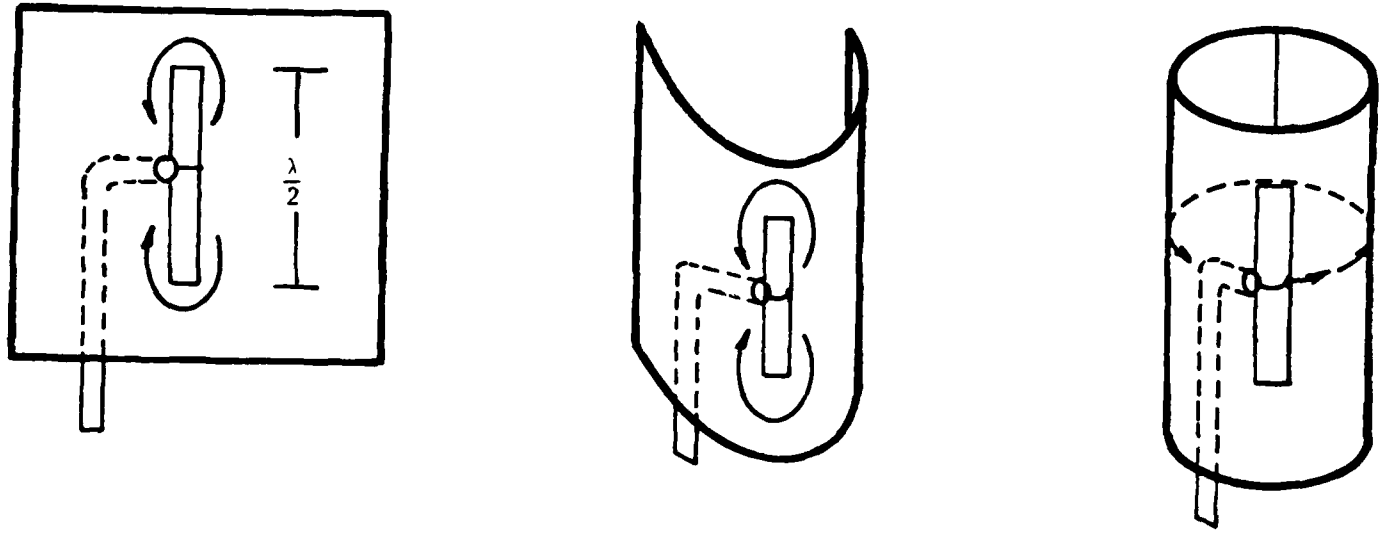

Figure 2. Formation of a slotted-cylinder antenna (4).

considered to be a slot in a flat metal sheet [4]. This slot is fed at its center with a coaxial cable, and the current directions are indicated by arrows. The sheet metal may then be formed into a U-shape and, finally, into a cylinder--with the coaxial feed cable inside the cylinder. If the impedance around the cylinder circumference is sufficiently low, current will flow in horizontal loops around the cylinder. Under these conditions, the slotted-cylinder functions as an antenna radiating a horizontally polarized field, the amplitude of which is dependent on the cylinder diameter. In general, the radiated field tends to be greater on the cylinder side where the slot is located; however, if the cylinder diameter is a sufficiently small part of a wavelength (approximately 0.1λ), the radiated field in the horizontal plane becomes essentially uniform. If the cylinder diameter is increased to the point of becoming a significant part of a wavelength, the field in the region of the shadow cast by the cylinder becomes small. Generally, as the cylinder diameter becomes large, the horizontal field approximates a cardioid [5].

On a previous program [2], the interactive relationship of slot width, slot length, cylinder diameter, and cylinder wall thickness was investigated; and the conclusion was made that a slotted-cylinder antenna, with the following features, would provide a suitable feed for the circular, parallel-plate waveguides:

- o cylinder diameter: 4 in.
- o cylinder wall thickness: 0.125 in.
- o slot length: 14 in., and
- o slot width: 0.125 in.

Four sets of circular, parallel-plate waveguides were stacked, one above the other, to provide a structure for exposing a large population of rats. An identical structure was provided to house the control rats. Because of the 12-ft diameter of the plates, 25 Plexiglas cages could be positioned around the inside periphery of each set of plates (Fig. 3) while a cage-center to cage-center spacing of 18 in. was maintained. The result of this separation distance was that intercage scattering of the exposure field was undetectable with small dipole probe antennas and a sensitive receiver tuned to the exposure frequency. The spacing between individual sets of plates in each structure was 18 in. because, at the 435-MHz exposure frequency, this distance was greater than one-half wavelength but less than one wavelength. This wavelength relationship assured that only the lowest order TE-mode propagated outward in concentric circles about the slotted-cylinder feed antennas located at the plate centers. The electric field vector, necessarily zero at the plate surfaces, prevented thereby the mutual coupling of fields between adjoining sets of plates. An extensive number of radiation pattern measurements were made, with the resulting data confirming TE_{01} propagation in which the vertical component of the electric field vector was typically 17 dB below the horizontal component (Fig. 4) at 435 MHz.

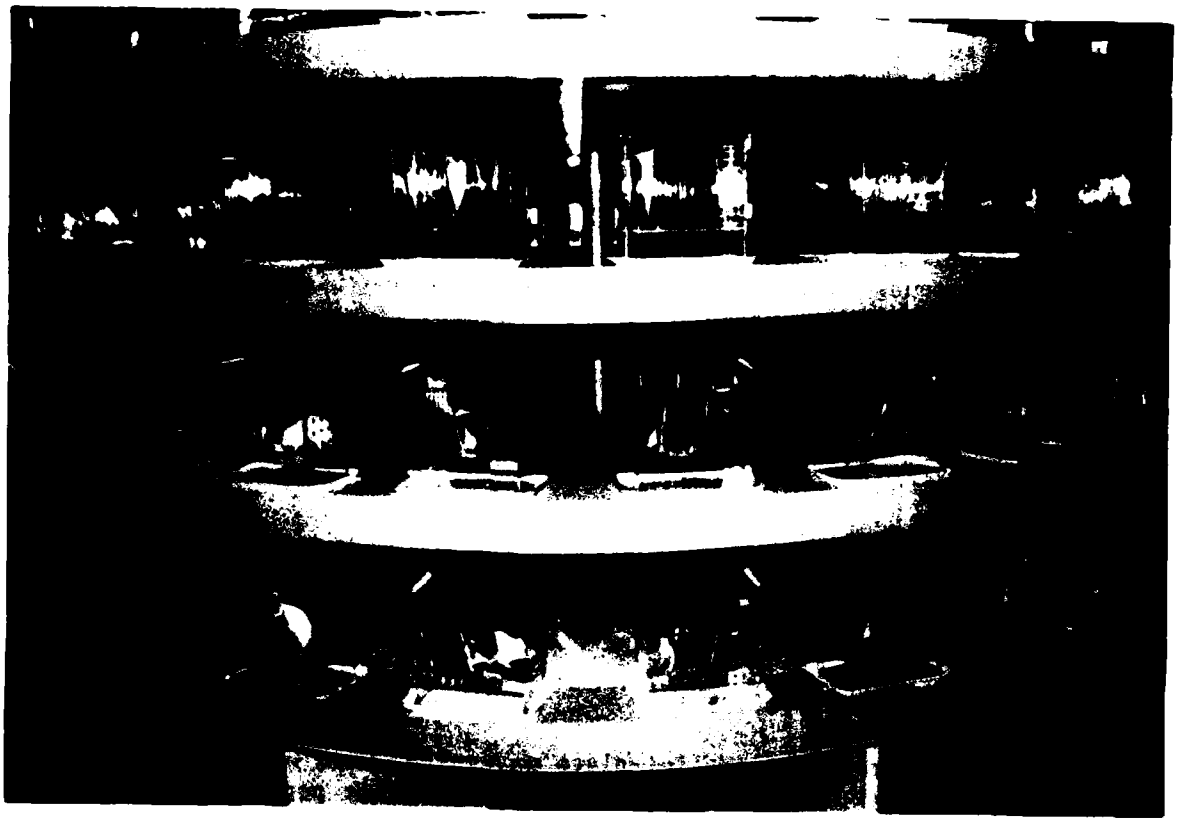

Figure 3. Rats positioned on circular parallel-plate waveguides.

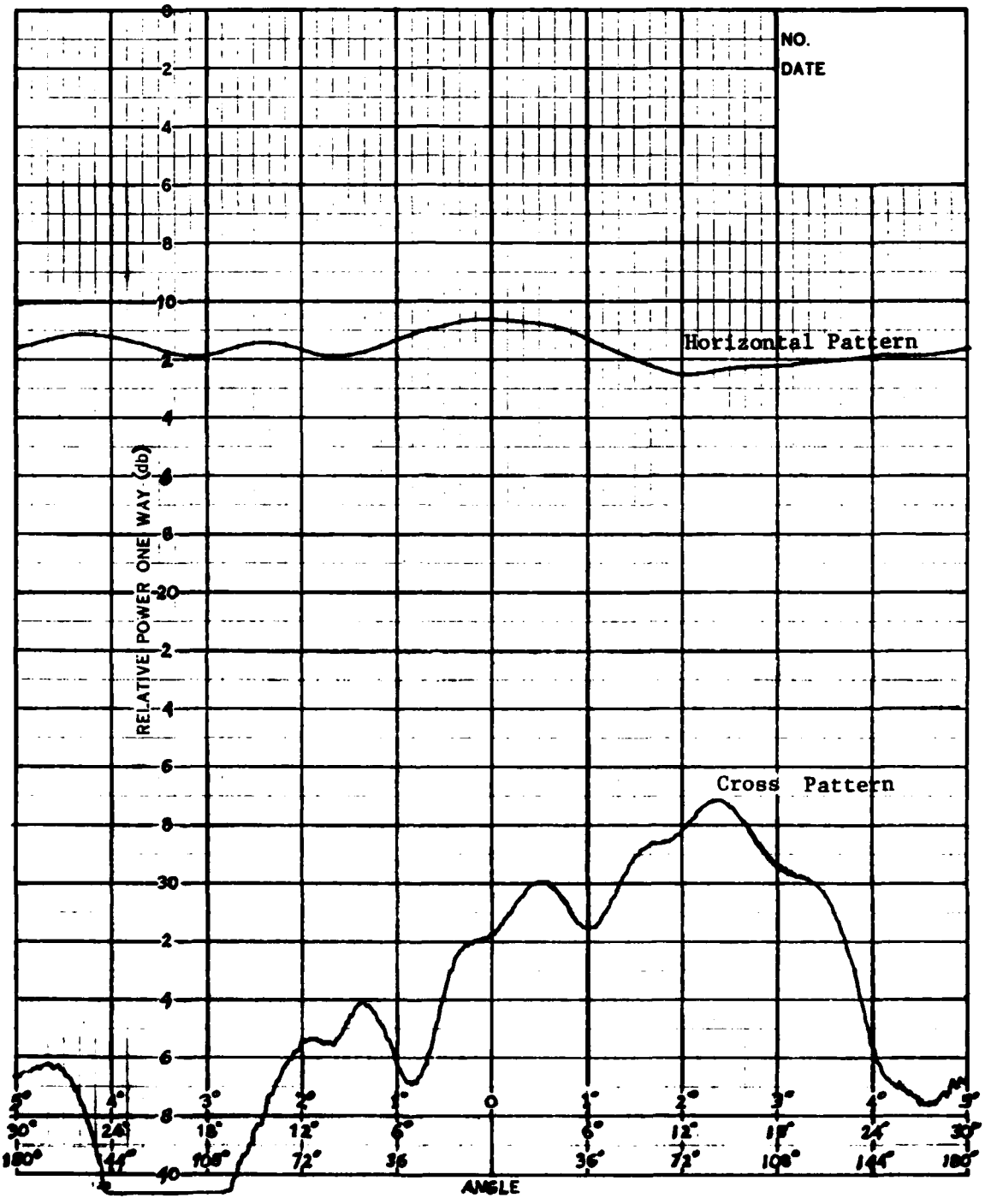

Figure 4. Typical radiation pattern, at 440 MHz, obtained with prototype, circular parallel-plate waveguide, and slotted-cylinder antenna.

During the shakedown evaluation, the transmitter was connected to slotted-cylinder antennas at levels 2 and 3 of the circular waveguides in the Radiation Room. The remaining two transmitter outputs were connected to dummy loads. The transmitter output was adjusted to provide a 1.0 mW/cm^2 exposure field at a position halfway between the plates at levels 2 and 3. This exposure field was measured with a Narda Model Probe, every 30 degrees around the plate circumference, to determine uniformity of the exposure field. As in previous measurements, the power density varied by less than ± 1 dB around the plates at both levels. These variations were reconfirmed in measurements made later with a 3-dimensional I-beam electric field probe on loan from the Bureau of Radiological Health [6]. With this probe, the signal-processing and display device were located outside the Radiation Room, and interconnection with the probe was provided by a fiber optic cable.

In brief, the RFR Facility provides space for eight contiguous rooms, along with independent heating, air conditioning, lighting, and water supply systems, plus open-ended waveguides for exposing and sham-exposing experimental animals. The RFR Facility is therefore an essentially self-contained area for conducting a wide variety of either long-term low-level, or short-term high-level bioeffects studies.

III. DATA ACQUISITION SYSTEM

In developing overall performance capabilities for the data acquisition system, everyone agreed that it must: (a) automate the data-logging procedure to the maximum extent possible; (b) monitor the status of the exposure fields; (c) provide a home alert, in case the exposure fields dropped below predetermined levels during nonwork hours; (d) perform basic statistical analyses of the data; and (e) interface with the Georgia Tech large mainframe computers for more extensive data processing. In conversations with USAFSAM personnel, we also agreed that the system should be compatible with those used in chronic bioeffects studies at USAFSAM and at the University of Washington. Compatibility with the USAFSAM and the University of Washington systems dictated a configuration around the industry standard S-100 bus. This standard, originally known as the "Altair" bus, appeared in the Micro Instrumentation Telemetry Systems (MITS) Altair computers in 1975. Because of its tremendous design flexibility, the Altair bus was quickly adopted by many microcomputer manufacturers; and it is now regarded as the most frequently used busing standard developed by the computer industry. Physically, the Altair bus consists of a set of 100-contact edge connectors mounted to a common motherboard and wired in parallel. Modules plugging into the edge connectors are circuit boards measuring 5 x 10 in.

With the foregoing system requirements in mind, a review of commercially available S-100 compatible computers was conducted, with the result that the Northstar Horizon and Cromemco System Two units were determined acceptable. Both offered a system core comprising: an S-100 motherboard; a Z-80 8-bit processor chip as the Central Processing Unit (CPU); a 64 K Random Access Memory (RAM); two drives for 5.25-in., double-sided, dual-density disks; a controller; a cabinet; and necessary power supplies. Retail prices for the Northstar Horizon and Cromemco System Two units were $4,330 and $4,695, respectively; however, both systems were available at discounts of approximately $750 from several different computer marketing centers. As capabilities of the two systems were compared in detail, we noted that either could be used for this program, but that the Cromemco System Two offered 390 kbytes per drive and a motherboard with 21 board slots. Comparable features of the Northstar Horizon system were 380 kbytes per drive and 12 board slots. On the basis of these differences in technical specifications, the Cromemco System Two unit was purchased; and the system configuration shown in Figures 5 and 6 was developed.

In the description of the data acquisition system shown in Figure 5, we first noted that the S-100 bus was originally designed for use with a CPU based on the 8080 microprocessor; however, in the Cromemco System Two, the CPU has been designed around the Z-80 microprocessor. Cromemco's designation for this Z-80 based CPU is ZPU. The ZPU in Figure 5 has the following technical specifications:

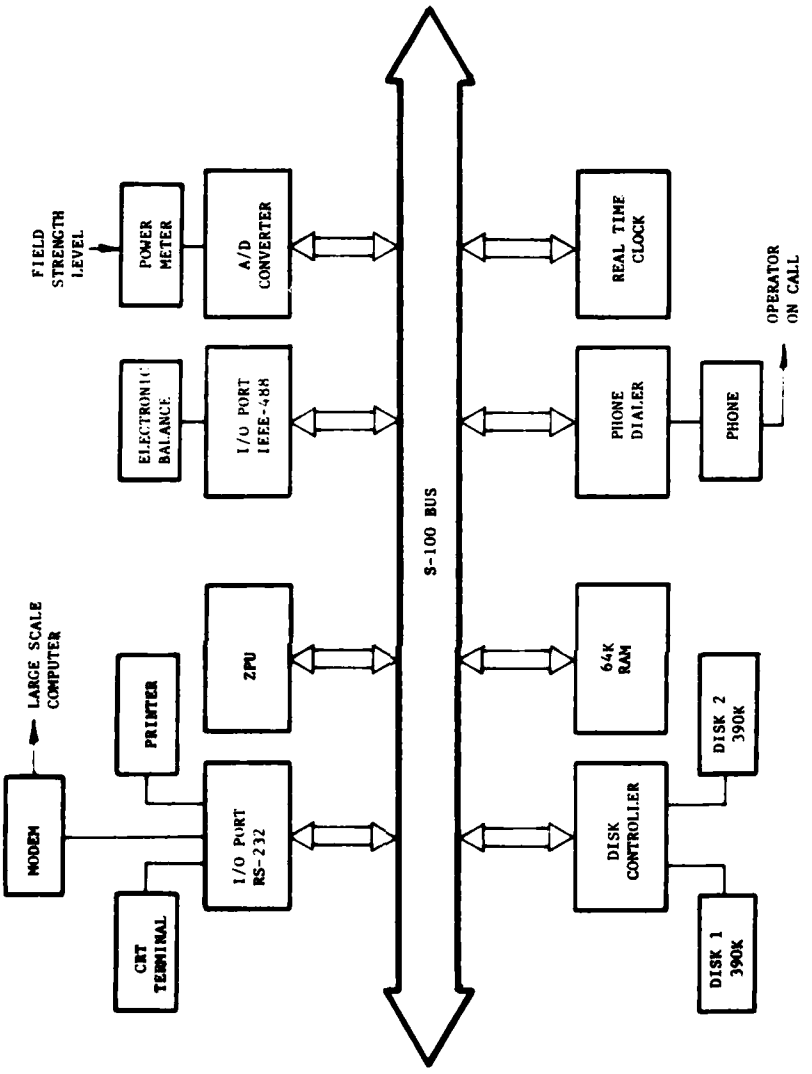

Figure 5. System configuration for Cromemco System Two Data Acquisition System.

Figure 6. Data acquisition system in the RFR Facility.

Processor: 4-MHz version of the Z-80 microprocessor

Clock Rate: 2 or 4 MHz (switch selectable)

Instruction Set: 158 instructions including the 78 instructions of the 8080 microprocessor

Power-On Jump: Jumper-wire enabled

Power-On Jump Locations: 16 switch-selectable locations

Wait-State Generations: 0-4 jumper-selectable wait states

M1 Wait State: Jumper wire selectable

Bus Compatibility: S-100

Power Requirements: + 8 VDC at 1.1A

The S-100 bus interfaces the Z-80 CPU module to as many as 20 additional memory, input/output (I/O) or other processor modules. Signals associated with this bus can be placed in four categories: (a) power supply; (b) address; (c) data; and (d) clock and control. The power supply signals involve three unregulated d.c. voltages: +8, +18, and -18 V. Since the main power supplies are unregulated, power supply regulation must be provided on individual circuit cards. For the address signals, 16 address lines allow direct addressing of 65,536 words of memory space. Tri-state transistor-transistor logic (TTL) drivers are used to drive the address bus. To handle data signals, the S-100 bus has two directional data buses, each eight bits wide. All clock and control signals are standard TTL levels and three clock signals are on the S-100 bus.

The 64-kbyte RAM is provided by a S-100 bus-compatible read/write memory board with the following specifications:

Memory Capacity: 65,536 Bytes

Memory Type: TMS 1416-15, 16K X 1 dynamic RAM

Memory Access Time: 250 ns (max)

Wait States at 2 MHz: None required

Wait States at 4 MHz: None required

Bus Compatibility: S-100

Power Requirements: +8, +18, and -18 V.

Interfacing of the CRT terminal, modem (for communicating with Georgia Tech mainframe computers), and printer with the S-100 bus is provided by a Cromemco D+7A Input/Output [I/O] Module. Specifications for this module are:

Analog Input Ports

Number: 7

Input Voltage Range: -2.56 to +2.54

Resolution: 8 bits

Conversion Time: 5.5 μs

Analog Output Ports

Number: 7

Output Voltage Range: -2.56 to +2.54

Resolution: 8 bits

Conversion Time: 5.5 μs

Parallel I/O Port

Input Port: 8 bits

Output Port: 8 bits

Input Load: one TTL equivalent

Output Drive: 10 TTL loads

Bus Compatibility: S-100

Power Requirements: +8, +18, -18 V

A Pickles and Trout Model P&T-488 interface permits the electronic balance to communicate with the S-100 bus. This interface appears as four I/O ports that are addressed as consecutive ports, with the first port address being an integer multiple of 4 (0,4,8,...). These ports allow the ZPU to manipulate the data, handshake, and bus management lines of the IEEE-488 bus.

The clock-calendar card shown in Figure 5 is a Scitronics, Inc., Model RTC-100 unit, which is used to provide the timing signals necessary for monitoring field strength in the Radiation Room on an interrupt-driven basis. This monitoring function also requires an analog-to-digital (a/d) converter to digitize the output of the power meter and communicate it to the S-100 bus.

To provide a hard copy of the data, the data acquisition system includes a Microline Model 82A Printer. This unit employs an impact dot matrix print system in which characters are a 9 x 7 matrix of dots. The printing direction is bidirectional; and the printing speed is 120 characters/sec, with the normal character spacings of 10 and 16.5 characters/in. The unit prints alphanumeric characters and symbols, plus lower-case English letters and symbols. A tractor unit is provided to feed paper into the printer. Interfacing of the printer with the S-100 bus is via a low-speed serial port based on the RS-232 code.

IV. CAGE WASHER SYSTEM

After technical specifications, cost, size, etc., of cage washers from several manufacturers had been reviewed, the Southern Cross Model 900-A Dyna Jet Washer was purchased and installed in Room D of the RFR Facility (Fig. 7). This washer, of stainless-steel construction, provides fully automatic wash, rinse, and final rinse cycles for all types of animal cages and accessories. A tempered safety-glass viewing window is installed in the washer front. Loading of the washer is accomplished, from the left (soil) side, through a guillotine pass-through door with a safety interlock feature that prevents washer operation until the door is closed. Exit is on the right (clean) side, through a pass-through door identical to the one on the entry side. Cage washing is provided by water jets from motor-driven, rotating, stainless-steel manifolds above and below the wash compartment. A 35-gal. detergent tank is below the wash compartment, and is used in the wash cycle. This tank contains a heavy-duty heating element with an external control, so that wash water temperature can be varied from room temperature up to 190°F. The rinse cycle uses hot tap water from the building utility supply. For final rinse, a 27-gal. tank with an automatic water-level controller is provided. External plumbing provides for the introduction of special final rinse fluids (distilled water, dionized water, etc.) and disinfectants.

Automatic reset timers control the wash and rinse time intervals as follows:

Wash Cycle = 0 to 10 min

Rinse Cycle = 0 to 10 min

Final Rinse Cycle = 0 to 1 min.

These timers are so wired that any cycle can be omitted by adjusting the cycle timer to the "off" position. After the timers have been set to the desired cycle durations, the start button is pushed and the wash operation is automatically continued through all cycles.

To facilitate wash and rinse operations, special purpose stainless-steel racks are provided for the Plexiglas cages and glass water bottles. Each of these racks hold 4 cages and 49 bottles in the wash compartment of the washer. The bottle rack provides a cover of stainless-steel mesh to prevent water jets in the washer bottom from forcing bottles out of the rack during wash and rinse cycles.

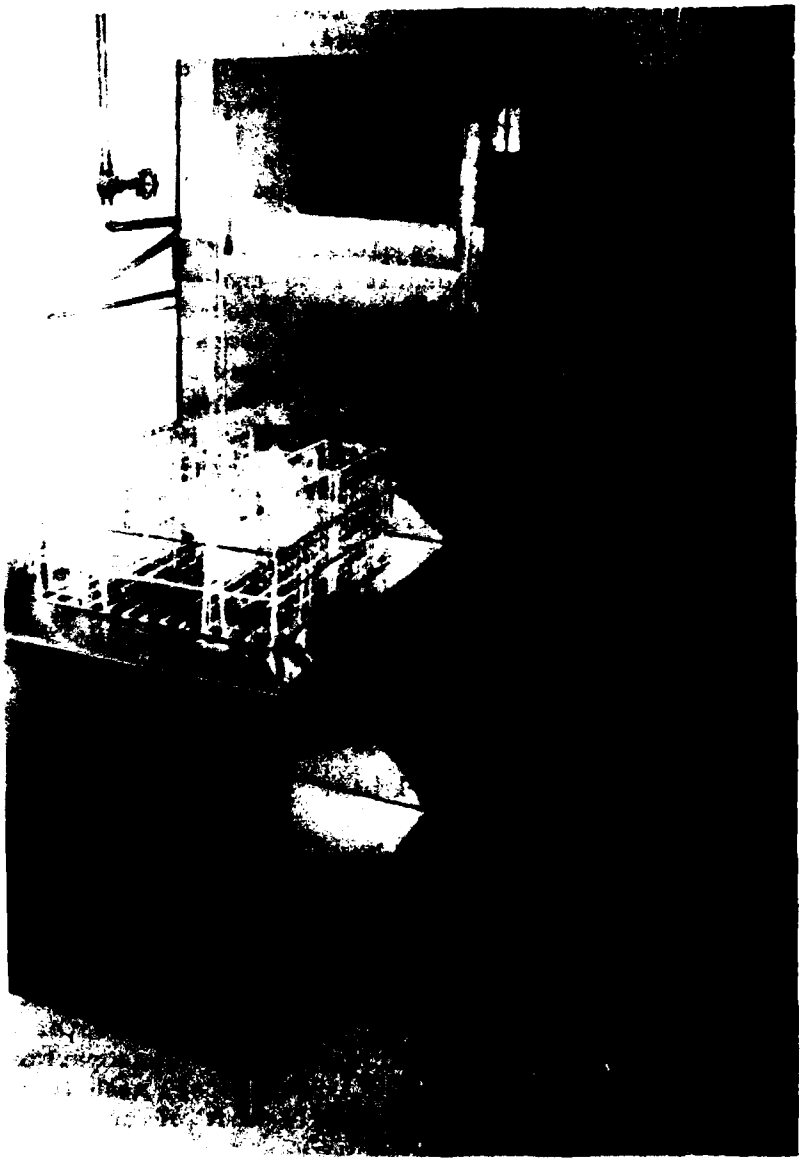

Figure 7. Cage washer in the RFR Facility.

V. ELECTRONIC BALANCE SYSTEM

Another important system purchased and installed during this program was the electronic balance. After the technical capabilities of balances from several manufacturers had been reviewed, the Sartorius Model 1203 MP Balance with built-in microprocessor, variable integration time, and locked-in readout was purchased. This balance offered a weighing range of 0 to 4000 g and readability of 0.1 g. Also purchased were a Sartorius Model 704201 Keyboard Programmer and a Model 705306 Cable box. The resulting electronic balance system was used to accurately weigh excited rats and automatically store the weight data in the data acquisition system. The keyboard programmer made it possible to program the balance for various different weighing procedures, including net weight, mean weight, average weight, accumulated weight, and difference from a predetermined average weight. The weight displayed by the balance was actually the average of a programmed number of individual weighings performed over either a fast (0.5-sec), normal (1-sec), or slow (2-sec) integration time. When the programmed number of weighings was completed, the average weight was locked in and displayed without fluctuation on a large, seven-segment digital readout. The taring capability of the balance was instantaneous and was accomplished when the tare switch on the balance front was touched. When the weight data displayed on the readout had achieved stability, a small "g" symbol appeared next to the weight value and indicated accurate data.

In most instances during the shakedown evaluation, the balance system was not interfaced with the data acquisition system because the wrong interface module was ordered. Late in the shakedown evaluation, the correct interface module was received; and the following program was used to weigh the rats accurately:

KEY	FUNCTION
g	Tare the balance Calls up stored regular weighing program
	Select weighing range on balance (0-4000g)
10	Key-in the desired number of individual weighings (in this case, 10)
CS	Store the desired number of individual weighings
25	Key-in rat identification number (in this case, number 25)
#	Transfer the number 25 to data output
	Place rat on balance pan

A Start program. The balance automatically weighs Rat No. 25 ten times, displays the number of individual weighings, stores and adds up the individual weight values, calculates the mean weight, displays the mean weight, and transfers the mean weight value to the data output.

Tare the balance

Place next rat on the balance pan

A Start program

VI. EXPERIMENTAL ANIMALS

All exposed and sham-exposed animals were carriers of chronic aortic cannulae. Implantation of the cannula was done under Nembutal anesthesia. The distal end of the cannula was positioned in the aortic arch (only 1-2 mm), while the proximal end was exteriorized at the back of the neck. Once implanted, the cannulae remain patent for months--some, for the rat's lifespan [7-8]. Over 16,000 rats have now been cannulated in Dr. Popovic's laboratory.

During this program, 150 Sprague-Dawley rats were used. The rats were delivered from the breeding colony (Harlan Sprague-Dawley, Madison, Wis.) to Dr. Popovic's animal quarters at Emory University. Being private quarters to Dr. Popovic's laboratory, the animal quarters are fully isolated. A rigid schedule of visual observation, care, maintenance, and cleanliness is enforced, resulting in ultraclean animal quarters and research laboratories. The lighting cycle in these quarters was 8 a.m. to 8 p.m., and the temperature was maintained at 24 + 1°C. In these animal quarters, the rats were provided food (Purina Rat Chow) and water ad libitum, and were carefully screened for health problems while growing to a body weight of 170 to 180 g. While growing to this weight, health status was monitored daily, and body weight was measured twice weekly. Rats that did not follow normal body weight curves (established for this strain during years in Dr. Popovic's laboratory) were eliminated, as were those with an increased total white blood-cell count or an unusual differential count (0.3 ml of arterial blood sampled once per week).

When the rats reached an approximate 175-g body weight, 75 of them were randomly selected for cannulation (all animals were cannulated by the same person: Biomedical Technologist C. Honeycutt, M.S.). For recovery from the cannulation, these rats were returned for seven days to their individual cages in the animal quarters. Fifty cannulated rats, in addition to 50 not cannulated, were randomly selected and then transferred to the RFR Facility at Georgia Tech. There, daily visual observations continued, and the rats were weighed twice weekly. In the RFR Facility, the rats were housed in Plexiglas cages, especially designed for this program, and positioned on the circular parallel-plate waveguides in the Control and Radiation Rooms, where the animals' acclimation to the new environment took place. A laboratory record was opened for each rat, and both environmental factors and health activity were placed in the animal's record. Neither the biomedical technologist who drew blood samples nor the assay personnel who analyzed the blood samples were able to distinguish between exposed and sham-exposed rats. Baseline data were obtained from the blood samples drawn and assayed for the biological endpoints proposed earlier.

VII. SHAKEDOWN EVALUATION

The shakedown evaluation was conducted to assure satisfactory completion of the numerous tasks associated with: (a) cannulating and managing a large population of rats; (b) operating the exposure facility; (c) drawing microsamples of blood; and (d) assaying the blood samples. The evaluation, conducted over an 8-week period, is described in the following paragraphs in terms of the biological endpoints monitored, the experimental procedures used, and the results obtained.

A. Biological Endpoints

The effects of long-term exposure to low-level RFR on rats and other mammals has been discussed in Georgia Tech Proposal No. EC-BR-1216: Long-Term, Low-Level Bioeffects Study Using 200 Cannulated Rats Exposed to 453-MHz Radiofrequency Radiation (pp. 7-11). This proposal concluded that, with a few exceptions in which artifacts were suspected, long-term exposure to low-level RFR does not appreciably alter biological parameters. This conclusion, of course, does not necessarily mean that all of the parameters stay unchanged; possibly some more sensitive or more reactive endpoints do change. Another possibility is that, after changing (which indicates a specific physiological disturbance), the endpoints revert to normal or near-normal levels because of adaptation on the part of the biological system.

Hormones are an example of endpoints likely to exhibit this behavior. The rationale for monitoring hormones is that exposure to RFR represents an environmental condition that might be stressful to biological systems. Certain humoral changes are known to be sensitive responders to stress. Of course, to detect the early beginning of such humoral change would be of interest, not only because of the crucial role of hormones in providing physiologic control of all vital processes, but also because such detection would provide an early clinical test to detect any possible harmful effect of RFR. The inter-relationship of hormones provides both the flexibility and the small gradations in response that are fundamental to homeostatic control. Hormones play a crucial integrative and regulatory role in such major processes as energy production, control of body fluids, growth, development, and others. Control of hormone secretion is especially important in maintaining homeostasis. Such control is achieved by a negative feedback mechanism, in which the hormone producing a biological effect achieves a sufficient magnitude to inhibit its further secretion. This negative feedback system consists of multiple components involving detection of a real or threatened homeostatic imbalance--a means of signaling secretion from secretory cells, responder organs or organ systems, and shutoff of secretion when adequate hormone levels are reached. By these mechanisms, the delicate physiologic balance required for homeostasis is maintained.

For this study, the biological endpoints chosen were: adenohypophyseal adrenocorticotropic hormone (ACTH); adrenal corticosteroid cortisosterone; catecholamines epinephrine and norepinephrine; growth hormone; and prolactin. Selection of these hormones was based on their common link to the existence of

exogenous stressors. This common link is evident when one reviews the literature dealing with possible effects of environmental stress on plasma levels of these hormones, and the possible mechanisms that might be involved. Also evident are some of the results obtained in stressed animals, and the importance of determining basal hormone levels for correct evaluation of the imposed stress. In the following paragraphs, some of the more significant research findings in this area are summarized.

A generally accepted concept is that catecholamines, ACTH, corticosterone, growth hormone and prolactin are good indicators of stress. This statement is true for man and for many animals, especially for rats (a species studied more than any other). The concept of stress has been widely accepted as a specific somatic response to damage, or threat of damage, by a wide variety of environmental agents. This concept was first suggested by the observation made in 1911 by Cannon and de la Paz. They showed that the adrenal medulla releases hormones in the cat during the emotional excitement associated with exposure to a barking dog [10]. In 1936, Hans Selye demonstrated evidence of a second endocrine system, the pituitary-adrenal-cortical axis [11]. This system, responding often to a more subtle psychic and physical stress, was able to induce a more global and profound influence on metabolic functions.

Other endocrine systems (in addition to those involving the adrenals) can respond to stimuli also. According to recent work, no endocrine system seems to be entirely free of the influences of stress. Despite these recent advances implicating virtually all endocrine systems, the central focus for present research has remained the altered pituitary and adrenal function in relationship to the handling of stress.

In this respect, rats have been studied more often than any other animal species. The literature shows that mainly Sprague-Dawley rats have been used, with Wistar rats having been used in a few cases. In most investigations hormone changes are assayed, in pre- and post-stress conditions, in plasma obtained after decapitation of the rats. In a few investigations, retro-orbital venous plexus punctures were used [12]. Blood sampling after cutting the tail was used more often [13]. In a somewhat larger number of investigations, the rats were bearers of venous catheters (PE 50, 51, 60, 61, 62) and were used a few hours or, at the most, one or two days after cannulation [10, 40]. Carotid artery cannulae were used more often [14-19] than venous cannulae, but also only a few hours (at most, a day or so) after cannulation. Sampling blood from semi-chronic cannulae is believed not to affect basal level of hormones; however, no studies show when animals have fully recovered from anesthesia and surgery. In one study [15], the level of catecholamines was found to be the same in arterial and in venous blood. Repeated collection of 0.5-ml blood samples--three times in 30 min (equaling 1.5 ml), or nine times in 12 hr (equaling 4.5 ml)--did not change the values of basal catecholamines [14]. This finding was difficult to accept. A decreased blood volume has been shown to increase catecholamine levels in plasma [20,21]. Blood used by some researchers was fresh; and, by others, frozen [22,23]. The plasma levels of "stress hormones" showed a clear

circadian (approximately 24 hr) rhythm [11,12]. Circadian increases of plasma corticosterone [24,25] were large, with perhaps a six-fold difference being observed between the lowest (early morning) and the highest (late afternoon or early evening) values [26].

The plasma hormonal levels in the rat were increased after stress [27-31], but plasma growth hormone level was decreased after stress [26,32,33]. Some hormones (for instance, ACTH) increased much after stress, sometimes ten-fold; but other hormones increased only slightly (e.g., corticosterone) [34]. Plasma prolactin was already increased 2 min after a mild stress [35]. A generally accepted concept is [36] that some hormones (for instance, catecholamines) respond to increasing magnitudes of stress in a step-wise fashion (i.e., respond monotomically), while others (for instance, corticosterone and prolactin) respond in an all-or-none fashion [27]. However, not all investigators agree with the second part of this statement. Some investigators felt that plasma corticosterone increased with increasing levels of stress in a step-wise fashion [37], just as do catecholamines. Hennessy and Levine were able to prove that "corticoid levels can sensitively reflect differences in the intensity of stimulation" [38]. The response of the pituitary-adrenal axis to stress [27-31] was striking. After short stress, the increased plasma level of hormones had a duration of about 30 min [39] or less, depending on the strength and duration of stress. Some investigations suggested that rats which had not been subjected to daily handling showed a more dramatic stress-increased serum prolactin level [27], and that training (periodic handling) lowered the level of "stress hormones" [14]; but according to other reports, handling (for four days in male rats) might increase prolactin response to stress [40].

For corticosterone, the biggest increase resulted from merely placing the rat in the test chamber [22]. Blood sampling in one rat affected the level of plasma "stress hormones" of other rats in the same room. Immobilization increased plasma catecholamines and corticosterone in conscious rats [17,37,41-46]. Stress increased plasma ACTH [47] and lead to pituitary-adrenal activation [48]. Even after a 2-hr immobilization, the plasma hormone values were still at the same high level as in the early beginning of the stress [49]. Repeated stress decreased reaction of "stress hormones" [50], thus causing "habituation." Values of plasma catecholamines and plasma prolactin were higher after decapitation than before, when blood was sampled from catheters [45,47]. Plasma growth hormone level (like adrenal steroids) has been found to be very responsive to stress [51-53], and falls rapidly after the stress. Probably growth hormone has circadian rhythmicity [51] which is inversely correlated with diurnal levels of corticosterone [23] and other hormones. However, not all investigators agree with the existence of circadian rhythm in growth hormone release [25,54] because of the inherent large variability of the data. Growth hormone is (and other stress hormones are) released in an episodic fashion in resting (nonstressed) rats. Thus, mean growth hormone levels exhibit characteristically high variability [55]. Besides the studied hormones, plasma renin activity [56] and gonadotrophins might also be useful indicators of induced stress. Of course, catecholamines are important controllers of renin release [57]; and, thus, catecholamine determination without renin activity measurements might be sufficient.

B. Experimental Procedures

During this program, experimental procedures were developed for monitoring hormone levels in a large population of rats exposed to pulsed-wave RFR at a frequency of 435 MHz. The hormone levels were monitored by assaying microsamples of blood drawn from the rats via their cannulae. The studied hormones responded to an exogenous stress in a manner similar to the dose-response relationship (i.e., the larger the stress, the greater the change in the plasma of stress hormones). Further, in order for changes in ACTH, corticosterone and prolactin to be studied, only 0.3 ml of arterial blood need be drawn from the chronic aortic cannula. No blood is wasted; a very small amount of blood is drawn weekly; samples consist of adequately mixed blood; the same rat can be used as its own control; and the rats can be followed before, during, and after RFR exposure lasting weeks or months.

On Monday, 26 April 1982, the rats were received at the Georgia Tech RFR Facility and began a two-week acclimation period. During this time, the temperature and humidity in the Radiation and Control Rooms were continuously monitored and recorded by using calibrated meters provided by the Physical Plant. A typical chart (Fig. 8) from one of the recorders shows that temperature and humidity were maintained at 72 $\pm$ 2^{o}F and 50 $\pm$ 2 percent, respectively. During this two-week period, rats from the exposure group were handled (placed in the small Plexiglas boxes) numerous times as part of their habituation process.

Two weeks after arrival at Georgia Tech, the rats were considered to have completed their acclimation period, and the shakedown evaluation with RFR began. Microsamples of blood were drawn from the cannulated rats on this day and on each of the following five Mondays, between 10:00 a.m. and 2:00 p.m. During these hours, the plasma levels of the measured hormones were at their lowest values. The experimental procedure involved deenergizing the 435-MHz pulsed-wave (pulse rate = 1000 pps, pulse rate = 1.0 μs, exposure power density = 1 mW/cm^{2}) transmitter, while a member of the program staff (animal caretaker) entered the Radiation Room with a cart designed to accommodate eight Plexiglas cages. (Only one staff member was authorized to work with the rats during procedures that involved the drawing of blood samples.) Eight numbered cages were removed from the circular parallel-plate waveguides, placed on the cart, and transferred to Room E (Fig. 1). When the staff member and rats were out of the Radiation Room, the transmitter was energized again. In Room E, the eight rats were transferred to small black Plexiglas boxes, just large enough to house an individual rat and provide a comfortable space large enough that the rat could turn around but didn't struggle to escape. The Plexiglas tops for these boxes were slotted so the cannulae could extend exterior to the box and be accessible for blood sampling. These small Plexiglas boxes were prenumbered to correspond to the numbers on the larger Plexiglas cages used to house the rats while in the Radiation Room.

Placement of the rats in the small Plexiglas boxes was done with slow, deliberate movements and in a manner that caused minimum excitement of the rats. When all eight rats had been transferred to the small Plexiglas boxes, they were then moved to Room F for a settling-down period of at least 15 min. Thereafter, the rats appeared to rest comfortably (to sleep, in most cases),

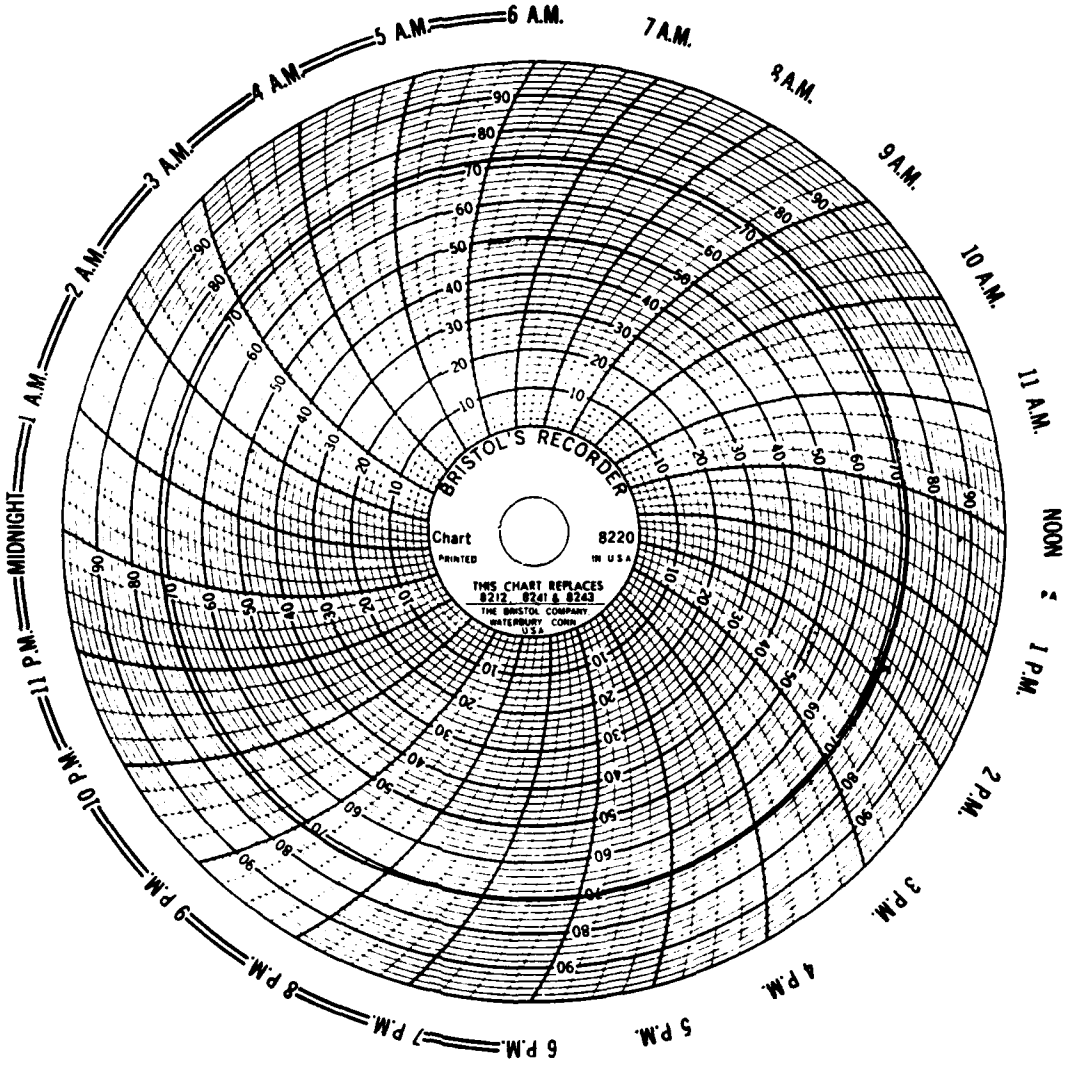

Figure 8. Temperature and humidity in the RFR Facility. (Chart)

and blood samples could be drawn during this resting period. Studies conducted during earlier programs have shown that this resting period extends for approximately 4 hr before the rats become restless. During this period (and the following period in which blood samples were drawn), only the biomedical technologist was permitted in Room F. Also, the door to the room was closed to prevent sounds from other areas within the Facility from exciting the resting rats. After 15 or more minutes, a 0.3-ml blood sample was drawn with the cannula protruding through the slot in the box top. Mosquito hemostats, padded with PE 240 plastic tubing, were then used to clamp the cannula slightly below its heat-sealed tip. These hemostats were used to open and close the cannula, as desired, during the remainder of the procedure. The heat-sealed tip was snipped off, and the animal's blood pressure slowly forced the heparinized saline solution and blood from the cannula. The heparinized saline solution (0.5-cc heparin--1000 units/ml from beef lung--per 30 ml of saline solution) was used to fill the cannulae at the time of their implantation. When the heparinized saline solution cleared the cannula tip, a 30-gauge needle attached to a 1-cc tuberculin syringe was used to draw 0.3 ml of undiluted blood. The drawn blood was then transferred to Sarstedt capillary collection tubes, calibrated for a 0.3-ml volume, and treated with EDTA to prevent clotting. These tubes were placed in a collection rack, submerged in crushed ice, where they were maintained until transfer to the assay laboratory. A different syringe and needle were then used to refill the cannula with heparinized saline solution, and heat was applied to seal the tip.

A new needle and syringe were used to draw blood from each rat. Also, a new needle was used to refill the cannula with heparinized saline solution after each blood drawing.

This procedure was repeated for each of the eight rats. The biomedical technologist then transferred the rats back to Room E. While blood was being drawn from the first eight rats, a second group of eight rats was removed from the Radiation Room, transferred to small black Plexiglas boxes and were ready to begin their settling-down period in Room F. As blood was drawn from this second group of rats, the first group was weighed with the electronic balance in Room E. The weight data were entered in the data acquisition system (described in Section V). After being weighed, the first group of rats was placed in clean Plexiglas cages and carefully returned to their marked positions on the waveguides in the Radiation Room; and a third group of eight rats was transferred to Room E, in preparation for having their blood samples drawn.

By repetition of this procedure, blood samples were drawn 6 times during the 7-week evaluation period for the RFR-exposed rats. Blood samples were not drawn during the fourth week because the biomedical technologist was ill. Weight data were taken 7 times from all cannulated rats. Other activities associated with the rats involved changing the Styrofoam soil trays under all cages every other day. Food hoppers and water bottles were checked daily, with food and/or water added as necessary. On Wednesday of each week, all cages were washed, by means of the washer (described in Section IV). All water bottles were washed on Friday of each week.

C. Experimental Results

Experimental results are presented as plots showing the variations in plasma ACTH, corticosterone, and prolactin for RFR-exposed and sham-exposed rats during the shakedown evaluation. Variations in growth hormone and catecholamines are not presented because, at the time blood samples were available, we lacked the specialized equipment needed for assaying plasma catecholamines and the specialized NIH kits needed for assaying growth hormone. Since then, the equipment and kits have been obtained and are available for follow-on studies.

Variations (mean values plus standard errors of the mean) in ACTH, corticosterone, and prolactin are presented as a function of time for exposed and sham-exposed rats in Figures 9, 10, and 11, respectively.

Sham-Exposed Animals. These animals were used primarily to determine:

- the number of times the animals should be handled in order to reach the true resting level at which the lowest plasma hormone values were reached. The literature, as already mentioned, strongly suggests that placing rats in new cages (boxes) induces rather high stress levels. The sham-exposed animals were therefore used during this study to determine the length of the habituation period (the number of times the rats should be handled and placed in the Plexiglas boxes used for blood drawing) necessary for our animals.

- whether the resting level of plasma hormones in our animals was comparable to levels measured by other investigations. This point was of particular interest, for the Plexiglas cages (used to house our animals) were of a specialized design, with a bottom made of glass rods spaced 0.5 in. apart. While such a rod separation was helpful, in maintaining cage cleanliness, it might have forced the animals to exert additional efforts in keeping their balance.

Results of these efforts indicate that, in the case of stress hormones, the first introduction to the Plexiglas box increased the plasma level of ACTH to that representing a mild stress. Plasma levels of corticosterone and prolactin were increased more. Two (corticosterone) or three (ACTH and prolactin) handlings of our animals were necessary before they reached true hormonal resting levels (Figs. 9, 10, and 11).

Exposed Animals. During the 2-week habituation period in new cages and new surroundings at the Georgia Tech RFR Facility, the rats (prior to RFR exposure) were placed twice in the Plexiglas holding boxes and left there approximately 20 min each time. The Week 0 data (Figs. 9, 10, and 11) represent the day the animals were placed for the third time in the Plexiglas boxes, blood samples were drawn for the first time, and RFR exposure was begun.

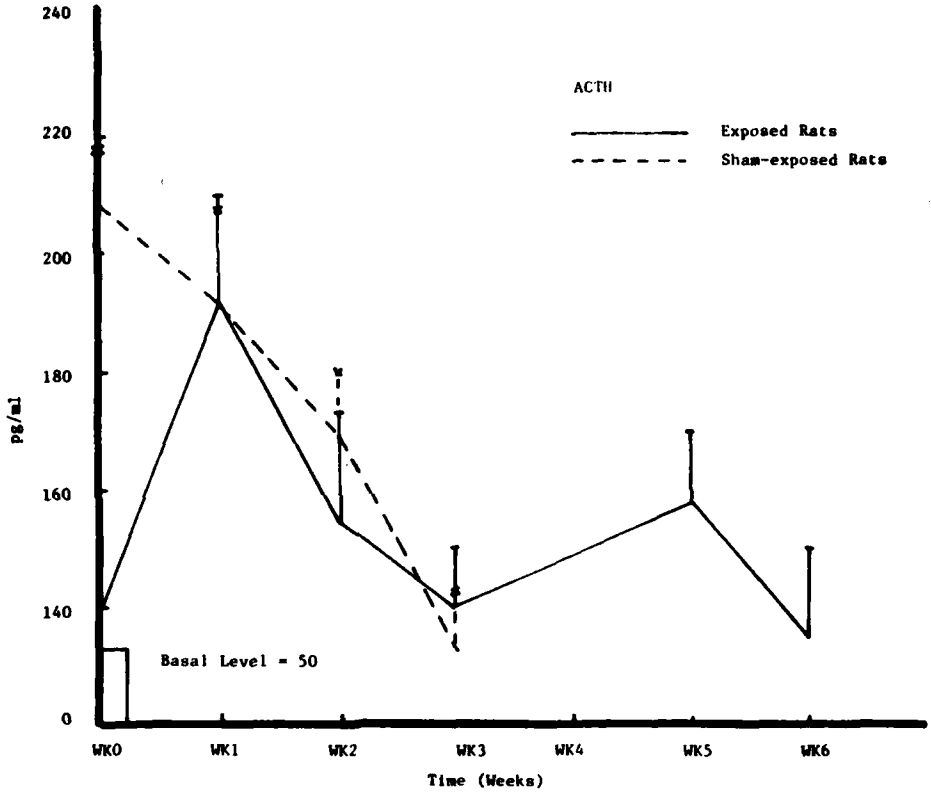

Figure 9. Variations in plasma ACTH levels.

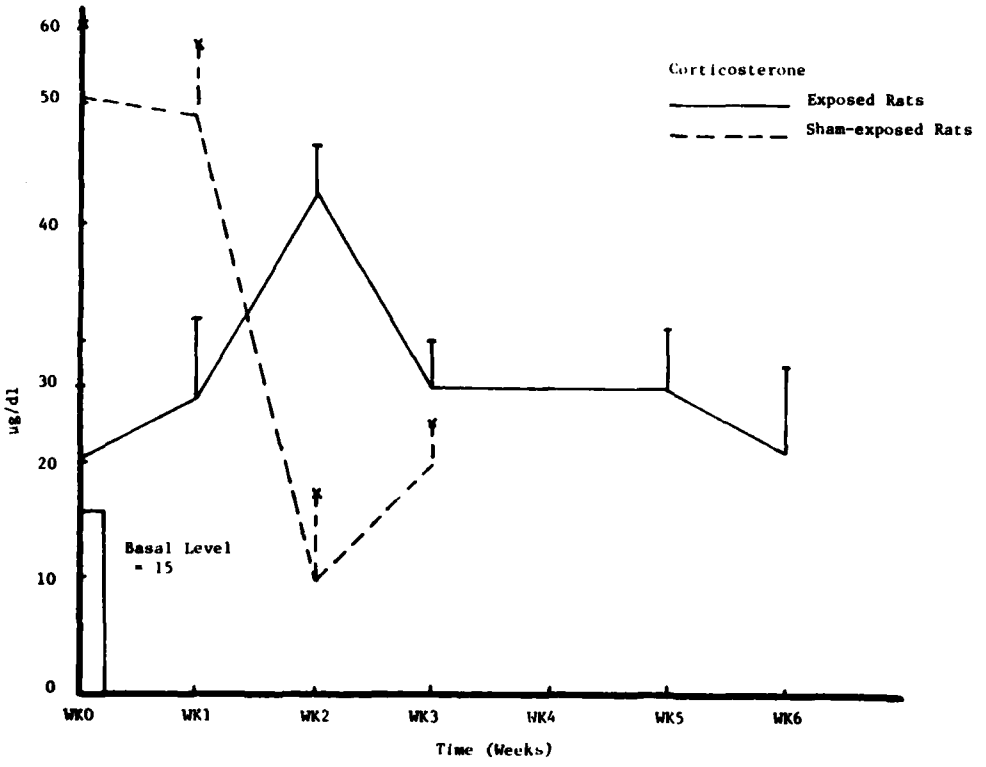

Figure 10. Variations in plasma corticosterone levels.

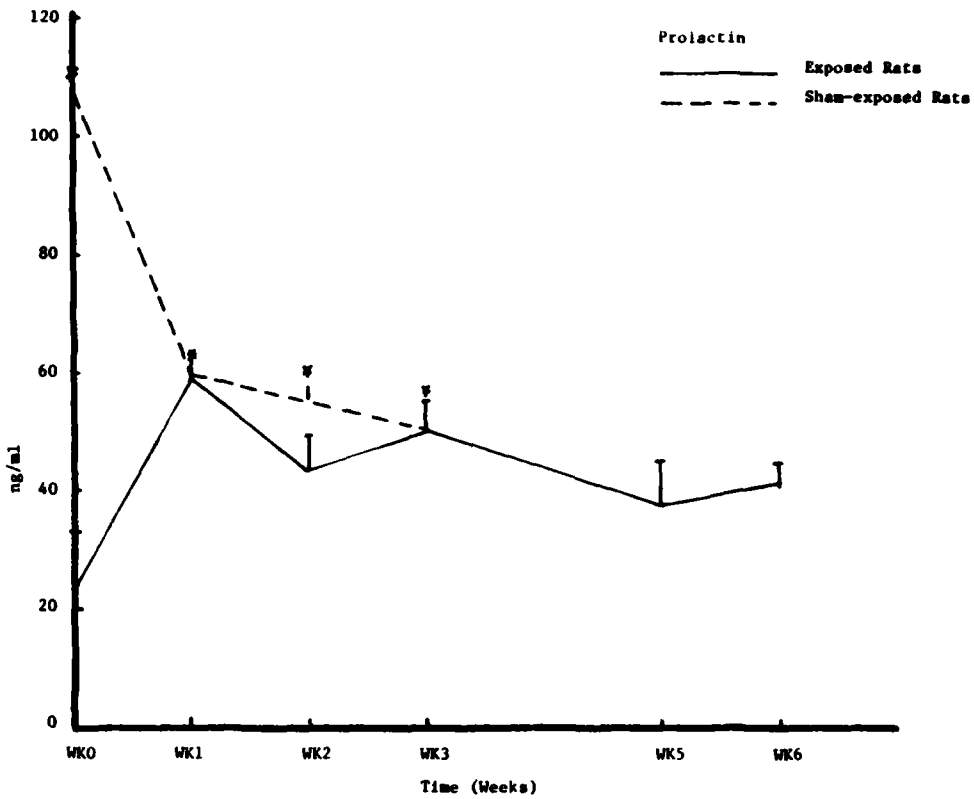

Figure 11. Variations in plasma prolactin levels.

Results show an increased plasma level for all three hormones, thus indicating an environmental stress possibly induced by RFR. The main peak for plasma ACTH occurred after one week of RFR exposure, and the corticosterone peak occurred at the second week. While these two hormones returned to preexposure or near preexposure levels one week later, the plasma prolactin level remained somewhat elevated throughout the shakedown evaluation.

In summary, the results indicate that plasma ACTH, plasma corticosterone, and plasma prolactin were increased during early exposure (first and second week) to low-intensity RFR. The "stress hormones" then returned to their resting levels, thus suggesting adaptation to the new environment.

Results of all plasma hormone levels were available 10 to 14 days after the blood samples were drawn. This length of time was found to be unacceptable, and the flow of results between the Departments of Physiology and Pathology will be expedited to provide results within no more than 4 days.

Basal hormone levels, as reported in the literature, are also plotted in Figures 9, 10, and 11. These levels are an approximate average of a number of values presented as basal levels for the conditions under which, and techniques by which, they were measured.

During the first 3 weeks of the shakedown evaluation, arterial blood was drawn from an additional 25 cannulated rats and radioimmunoassays were performed. This blood was drawn on Monday of each week; and two samples, rather than one, were drawn 20 minutes apart while the rats remained in the Plexiglas holding boxes. Plasma hormone levels were similar in both samples for each rat, thus demonstrating the adequacy and reproducibility of the radioimmunoassay techniques. Furthermore, the techniques of double blood withdrawal indicated that the main "stress" which elevated plasma hormones at the initial week was exposure to an unfamiliar holding box (lasting 20 min or, as in the case of second blood withdrawal, 40 min) and, to a lesser degree, removal from the cage. When the values of plasma hormones were decreased in Week 2 (third week of sampling), the difference between two samples was smaller; but, again, the second sample did not show any decrease in values.

Weight data for the exposed and sham-exposed rats are presented in Figure 12. The sham-exposed rats entered the evaluation at a mean weight slightly less than that of the exposed rats. This difference was maintained throughout the evaluation without statistically significant variations, as determined by the Student t-test (p-values greater than 0.5 in each case). Comparisons of these data with weight data from other rats are not presented, because no growth curves exist for cannulated rats housed in anything approaching the specialized Plexiglas cages used during this program.

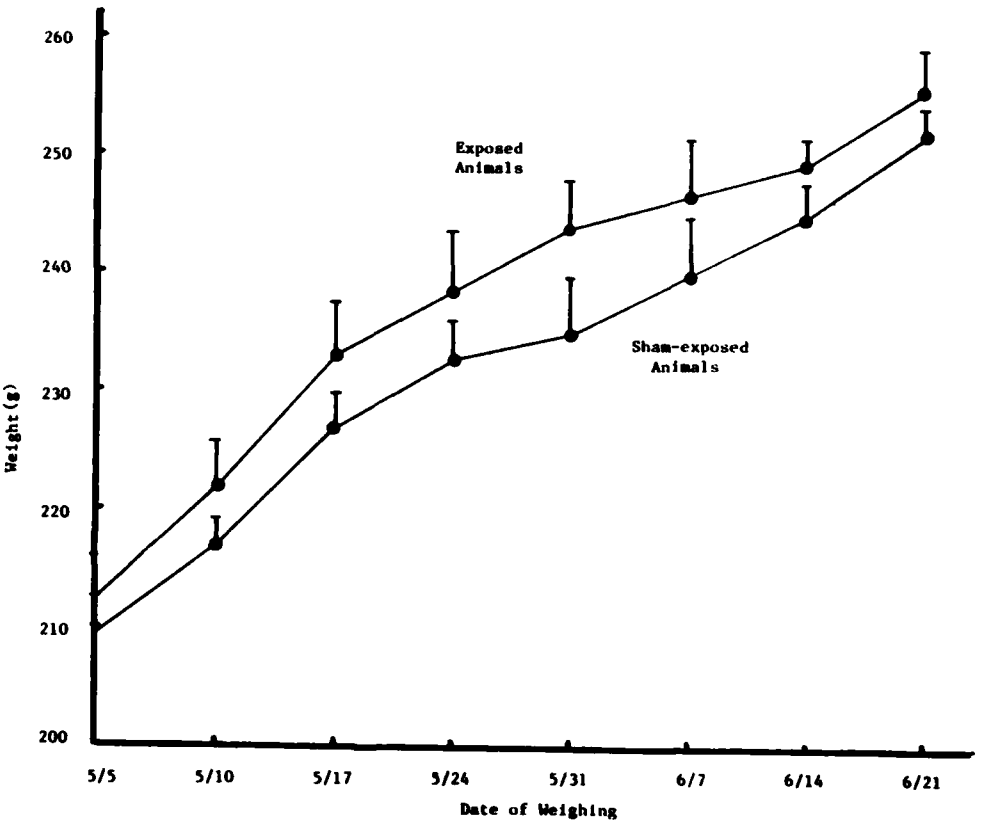

Figure 12. Weight for exposed and sham-exposed animals during the shakedown evaluation.

VIII. CRITIQUE

The overall purpose of this program was to conduct a six-week operation (shakedown evaluation) of the 435-MHz RFR Facility to assure that all tasks, associated with a comprehensive long-term bioeffects study involving a large rat population, could be adequately accomplished. In the subsequent proposal for the first long-term bioeffects study (Proposal No. EC-BR-1216: Long-Term, Low-Level Bioeffects Study Using a Large Rat Population Exposed to 435-MHz RFR, submitted to USAFSAM/RZP, 25 May 1982), a number of procedures based on results of the shakedown evaluation were incorporated. Now, several additional modifications can be proposed on the basis of new data collected after the proposal was submitted. These modifications are presented as a critique of procedures used during this (shakedown) program, and as recommended changes to these procedures:

- The Plexiglas cages, especially designed to house rats in the Radiation and Control Rooms, apparently used too few glass rods to form the floor. These rods were spaced 0.5 in. apart, and possibly provided inadequate support for the rats. This problem resulted in a longer habituation period for the animals than would have otherwise been necessary, and in plasma hormone values above BMR levels. Now, the number of rods in the floor has been doubled in experimental cages being used to analyze the floor support and its influence on the habituation and plasma hormone levels of the rats. This analysis is expected to indicate a need for improved flooring in cages in follow-up studies.

- Persons involved in handling the rats and maintaining the Facility during this shakedown evaluation were not professionals working full-time on the program. Follow-on research programs will include a full-time person professionally trained as an animal caretaker, who should be able to handle large numbers of rats without inducing undue excitement in them. All animals will be handled by this person only. In handling each rat, the caretaker will wear a heavy glove; and his gloved hand will be placed underneath and around the animal's body.

- Both RFR-exposed and sham-exposed animals will be handled (placed in the Plexiglas holding box for 20 min) at least four times before initiation of the radiation.

- Microsamples of blood will be drawn from all RFR-exposed and sham-exposed animals three times before initiation of the radiation. Only after hormonal levels have fully stabilized will the actual study begin.

- Microsamples of blood will be drawn twice (Day 2, and Day 5) during Week 1 and Week 2 after initiation of radiation. Assay results from these blood samples will better define changes in plasma hormones during a time of possible acclimation.

- Cannulae will be sealed with a removable plastic plug (not heat-sealed) that permits much longer use of the original cannulae and requires no extension parts.

- All animals will be completely undisturbed for 18 hr prior to blood sampling.

- As explained in Section VII.C ("Experimental Results"), the NIH kit for radioimmunoassay of rat growth hormone was not available during this program. We have now obtained the necessary kits with highly purified porcine growth hormone as the ^{125}I labeled antigen. Radioiodination of growth hormone will be carried out according to the methods established by D. Heber et al. [58]. For rat growth hormone assay, reagents are not commercially available. Reagents routinely used in clinical laboratories for human growth hormone determination by radioimmunoassay are not usable because of cross-reactivity of rat growth hormone with corresponding human hormones.

- Active efforts are underway to implement a liquid chromatographic assay for plasma catecholamines. In the past, experiments were made with a radiometric technique routinely used in the clinical laboratory for assaying catecholamines in human plasma. The technique involved several chromatographic steps, etc., which made the experimentation both tedious and time consuming. Further, the technique required 0.5 ml of plasma, which could not possibly be scaled down. High performance liquid chromatography with electrochemical detection (Oka et al. [59]) offers several advantages over previous techniques (fluorometry, bioassay, calorimetry, and radioassay). This method is available for urine and brain tissue catecholamines, and will be adopted for plasma. Indications are that this method can be scaled down to require only 50-µl samples.

- All assays will be performed under strict quality control rules. The same guidelines (controls, split samples, blind samples, etc.) will be applied as in the clinical laboratory of Emory University Hospital. Participation will continue in evaluation programs such as those sponsored by the Center for Disease Control, the Association of Clinical Chemists, and the College of American Pathologists.

IX. REFERENCES FOR SECTIONS I-VIII

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